Insulin-like growth factor system in human central nervous system, multiple sclerosis and amyotrophic lateral sclerosis

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Chapter 2

Insulin-like growth factor-I receptors in human brain and pituitary gland: An Autoradiographic study

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Abstract

Insulin-like growth factor (IGF)-I receptors were studied in adult human post-mortem brain and pituitary gland using quantitative autoradiography with human recombinant [^{125}I]IGF-I. The highest densities were found in the choroid plexus, pituitary gland - where IGF-I receptors were mainly concentrated in the anterior lobe, pineal gland, glomerular layer of the olfactory bulb, and the molecular layer of the cerebellar cortex. Moderate densities were present in cerebral cortex, caudate nucleus, putamen, accumbens, the CA1, CA2, CA3 fields and dentate gyrus of the hippocampus, the dendate nucleus of the cerebellum, amygdala, thalamus, pontine nuclei, and substantia nigra. All other brain areas, including white matter, contained low densities of IGF-I receptors. The finding that several well-defined brain structures are enriched with IGF-I receptors suggests a neurotrophic/survival or neuromodulatory role of insulin-like growth factors on specific neuronal systems. IGF-I receptors observed in the white matter may be associated with oligodendrocytes.

Introduction

Several lines of evidence suggest that insulin-like growth factors (IGFs) I and II, originally named somatomedins and structurally related to insulin, play an important role in the development and growth of the nervous system [3]. They can increase DNA synthesis in rat foetal brain cell cultures [13] and astroglial cells [9], enhance the survival of cultured neurons [24] stimulate neurite outgrowth [20], and stimulate the development of oligodendrocytes and myelin synthesis [2, 17, 18, 19, 21]. Transgenic mice that overexpress insulin-like growth factor-I (IGF-I) develop larger brains that contain more myelin than controls [6]. An elevated IGF-II content in cerebrospinal fluid has been observed in a child with megaencephaly [26]. IGFs are also present in the mature rat and human brains [5, 10, 16]. A variant N-terminally truncated form of IGF-I, IGF-II, and higher molecular mass forms of IGF-II, have been detected in adult human brain [5, 10]. However, at present, nothing is known about their functional role(s).

Two types of IGF receptors have been identified: IGF-I and IGF-II receptors [8, 25]. Studies on cell cultures indicate that the proliferative effects of both IGF-I and IGF-II are mainly mediated via the IGF-I receptors [3]. The IGF-I receptor is a tetramer composed of two alpha and two beta subunits linked by disulphide bounds; the alpha subunit contains the ligand binding site and the beta subunit consists of a transmembrane domain and a cytoplasmic tyrosine kinase domain [27]. The distribution of IGF-I receptors has been studied in detail in adult rat brain [4, 14]. Only two studies aimed at demonstrating IGF-I receptors in adult human brain have been reported. The first study was performed on post mortem-obtained brain tissue homogenates from a single individual, and revealed the presence of IGF-I receptors in various brain regions [25]. The second study utilised autoradiography on large whole-hemisphere cryosections from two male subjects [1]. Because of this technique, the exact localisation of the IGF-I receptors in several regions of the human brain could not be determined, and many brain regions were not investigated.

In order to obtain a more detailed regional distribution of IGF-I receptors in adult human brain we applied quantitative autoradiography with human recombinant [^{125}I]IGF-I on microscope slide-mounted sections.

Material and methods

Tissue preparation

The use of human brain tissue was approved by the ethical committee of the Free University of Brussels. Brains and pituitary glands were obtained from 5 patients, without neurologic or psychiatric disease (one female of 73 years, and four males of respectively 52, 47, 66, and 77 years). Post mortem delay, defined as the time elapsed between death and freezing of the brain sections, ranged between 4 and 12 hours. Causes of death were sudden cardiovascular arrest (1), pulmonary oedema (1), cancer (2), and hematemesis (1).

Blocks of tissue of approximately 0.5 cm thick were dissected from coronal brain cuts at 0-4°C, frozen rapidly by immersion in isopenthane-dry-ice, and stored at - 80°C until used. The frozen blocks of tissue were mounted on a cryostat chuck coated with plastic embedding medium (Bio-Optica, Milan, Italy), and serial sections of 10 μM thickness were cut at -20°C in a cryostat (Reichert-Jung cryostat Frigocut 2800), thaw-mounted on gelatine-coated glass slides and dried.

Binding experiments with [^{125}I]IGF-I

Frozen sections of the cerebellar hemisphere were used to determine the optimal conditions for autoradiographic labelling. The sections were preincubated for 15 min at 25°C in 25 mM Tris-HCl (pH 7.5), containing 10 mM $MgCl_2$, and 0.1 % bovine serum albumin. Binding experiments were done in duplicate at 25°C in the same buffer composition. Association experiments were performed by incubating the sections for increasing periods of time with 0.1 nM human recombinant [^{125}I]IGF-I. For saturation binding studies the sections were incubated for 60 minutes with [^{125}I]IGF-I using concentrations between 0.01 and 2 nM. Competition binding experiments were done with 0.1 nM [^{125}I]IGF-I. After incubation, the sections were washed 3 times for 1 minute each in the same buffer to remove unbound ligand, and then quickly dipped in distilled water. The sections were then wiped from the slides using Whatman GF/B glass fiber filters, and radioactivity was determined in a gamma-counter.

Non-specific binding was determined in the presence of 0.5 μM unlabelled IGF-I. Specific binding to IGF-I receptors was obtained by subtracting the non-specific binding from the total binding. Binding isotherms were analysed by non-linear least-square curve fitting.

The K_D value of [^{125}I]IGF-I was calculated by linear regression analysis of the Scatchard plots. The dissociation constants (K_I values) of the competitors (IGF-I and IGF-II) were calculated from the corresponding IC_{50} (inhibitionconcentration) values by the method of Cheng and Prusoff [7].

Autoradiography of IGF-I receptors

Consecutive frozen sections from the different brain regions were preincubated for 15 min at 25°C in 25 mM Tris-HCl (pH 7.5), containing 10 mM $MgCl_2$, and 0.1 % bovine serum albumin. The sections were then incubated for 60 minutes at 25°C in the same buffer with 0.1 nM [^{125}I]IGF-I. Autoradiograms representing non-specific binding were produced by incubating serial sections under identical conditions with 0.5 μM IGF-I.

After incubation, the sections were washed 3 times for 1 minute each in the same buffer to remove unbound ligand, and then quickly dipped in distilled water. The sections were dried under a stream of cold air, placed in X-ray cassettes together with commercially available [^{125}I]standards (Amersham, Gent, Belgium), and exposed to [^{3}H]Ultrofilm (Amersham) for 4 days. The films were developed with a D19 Kodak developer at 4°C, and after drying they were placed on a light box where they were scanned by a video camera. The images obtained were digitised, analysed and quantified by computer-assisted densitometry using the program Image (National Institutes of Health Research Services Branch, NIMH, Betheseda, MD). A calibration curve was generated by fitting of optical density and disintegration's per minute per milligram polymer values of the [^{125}I]standards. The regions of interest were sampled and mean optical densities determined, and converted into fmol/mg protein, based on the experimentally determined relation between polymer and brain paste standards. Brain areas and nuclei were identified using the human brain atlas of Nieuwenhuys et al.,[22]. Specific binding values were obtained by the subtraction of non-specific binding images from corresponding total binding images.

Materials

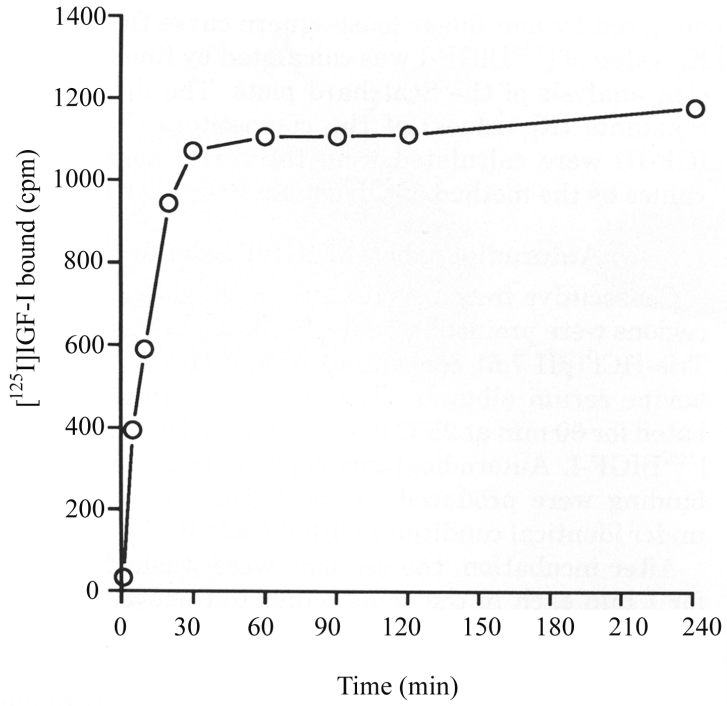
[^{125}I]IGF-I was obtained from New England Nuclear (Brussels, Belgium). IGF-I and IGF-II were purchased from Boehringer Mannheim (Mannheim, Germany). [^{125}I]standards and [^{3}H]Ultrofilms were obtained from Amersham (Buckinhamshire, UK). All other chemicals were of the highest grade commercially available.

Results

Characteristics of the binding of [^{125}I]IGF-I

To determine the optimal incubation conditions for the autoradiographic experiments, [^{125}I]IGF-I association, saturation and competition binding studies were performed on slices of cerebellar hemisphere. Binding of 0.1 nM [^{125}I]IGF-I reached equilibrium within 60 minutes (Figure 1), and this incubation time was adopted in subsequent experiments.

Figure 1

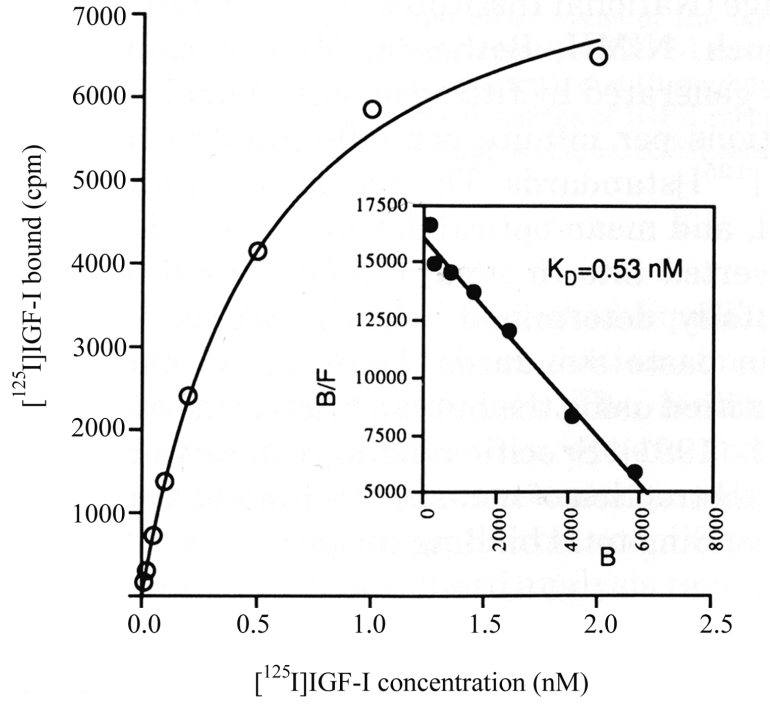


Time course of 0.1 nM [^{125}I]IGF-I specific binding to slide-mounted microtome sections of human cerebellar hemisphere. After incubation at 25°C for increasing time intervals the sections were wiped from the slides using Whatman GF/B glass fiber filters, and radioactivity was counted in a gamma-counter. Specific binding was calculated by subtracting non-specific binding, determined in the presence of 0.5 μM unlabelled IGF-I, from total binding. Equilibrium was reached within 60 minutes (min).

The binding of [^{125}I]IGF-I to human cerebellar hemisphere sections was saturable indicating a finite receptor population. A representative saturation isotherm and Scatchard plot is shown in Figure 2. Scatchard analysis revealed a K_D value (mean ± S.E.M, n=3) of 0.52 nM ± 0.04 nM.

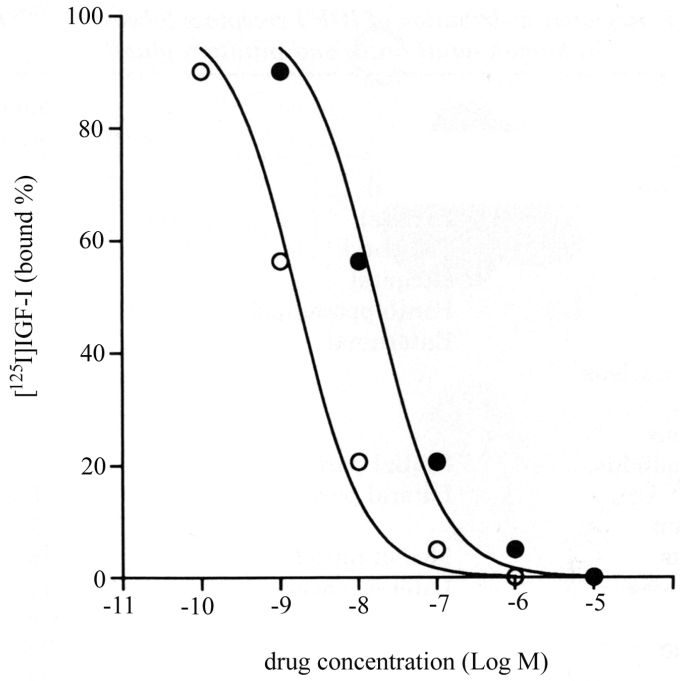
Representative [^{125}I]IGF-I/ IGF-I and IGF-II competition curves are shown in Figure 3. The curves were best fitted to a one-site binding model, and the calculated K_I value (mean ± S.E.M, n=3) was 1.4 ± 0.4 nM for IGF-I and 18.2 ± 2.5 nM for IGF-II. A concentration of 0.5 μM IGF-I produced maximal displacement of [^{125}]IGF-I binding, and this concentration was used to determine non-specific binding in the autoradiographic experiments.

Figure 2



A representative saturation isotherm for [^{125}I]IGF-I specific binding on microtome sections of human cerebellar hemisphere. The sections were incubated with increasing concentrations of [^{125}I]IGF-I at 25 ºC for 60 min, and radioactivity was determined as described in Figure 1. **Inset:** Scatchard plot of the specific binding

Figure 3



Representative competition curves for IGF-I (o) and IGF-II (●) inhibition of 0.1 nM [^{125}I]IGF-I specific binding on microtome sections of human cerebellar hemisphere. The incubation conditions are the same as in Figure 2. Computer analysis of the binding data revealed that the competition curves were best described by a one-component binding model. K_I values were 1.4 nM for IGF-I and 12 nM for IGF-II.

Regional distribution of [^{125}I]IGF-I receptors

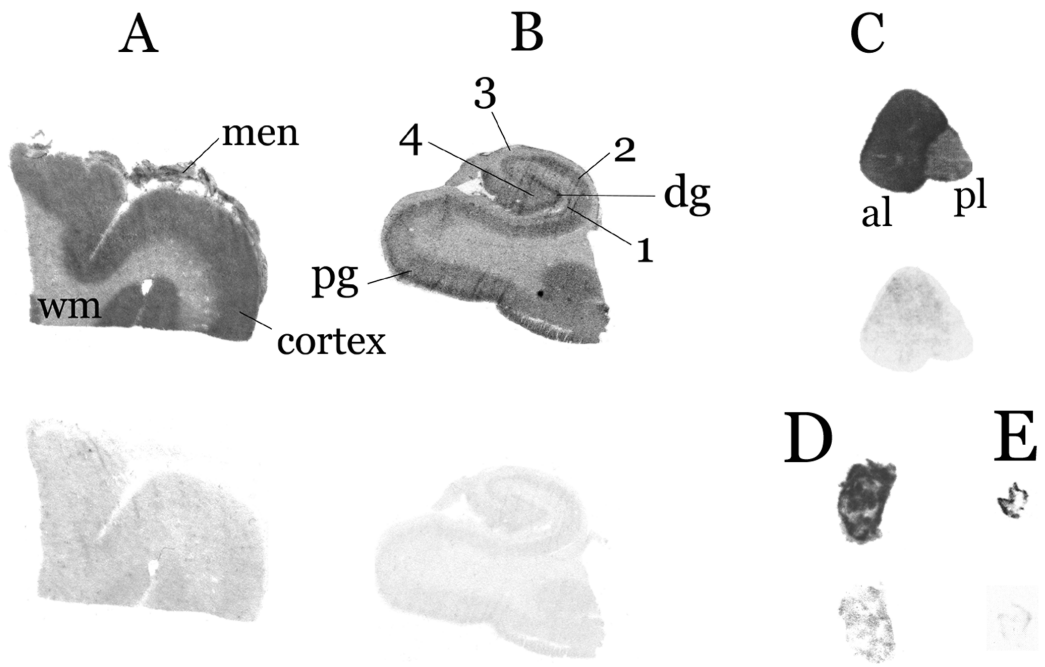
[^{125}I]IGF-I labelling showed a widespread distribution of IGF-I receptors throughout the human brain and pituitary gland. The regional distribution is summarised in Table 1.

The highest levels of IGF-I receptors were found in the pituitary gland, predominately in the anterior lobe but also in the posterior lobe (Figure 4C), choroid plexus (Figure 4D), and pineal gland. The cerebral cortex contained a moderate density of IGF-I receptors, without a distinct laminar distribution (Figure 4A). Similar densities were found in the claustrum (Figure 6B).

In the hippocampal formation, high levels of IGF-I receptors were seen in the dentate gyrus and over the pyramidal layers of the CA1, CA2 and CA3 fields (Figure 4B).

In the olfactory bulb, IGF-I receptors were especially concentrated in the glomerular layer (Figure 4E). Slight labelling of more or less uniform density was found throughout the white matter of the cerebral and cerebellar hemispheres, and brainstem.

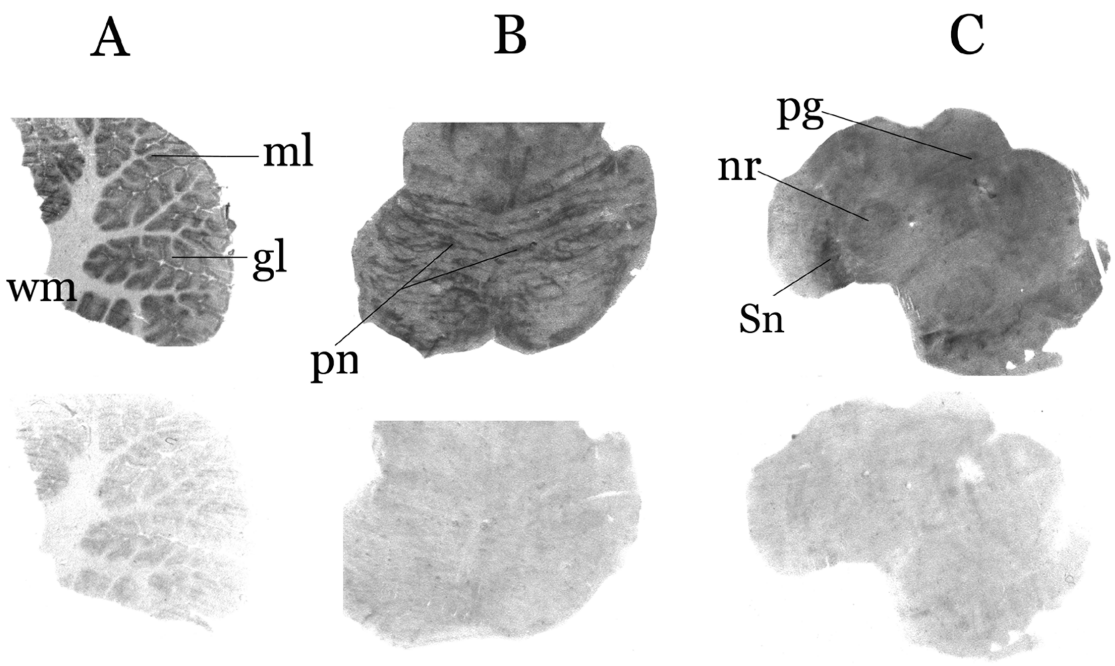
Figure 4



Autoradiographs of 0.1 nM [^{125}I]IGF-I binding in the absence = total binding (top row) and the presence of 0.5 μM unlabelled IGF-I = non-specific binding (bottom row) to microscope slide-mounted sections. **A:** Distribution of IGF-I receptors in frontal cortex/white matter. In the frontal cortex, and other areas of the cerebral cortex (not shown) there is no laminar distribution of IGF-I receptors. Note the diffuse labelling in the meninges (**men**), and throughout the white matter (**wm**), corresponding to low densities of IGF-I receptors. **B:** Distribution of IGF-I receptors in the hippocampus. IGF-I receptors are concentrated in the pyramidal cell layers of the CA1, CA2 and CA3 fields (**1, 2, 3**) and the dentate gyrus (**dg**). Lower levels are present in CA4 (**4**). Moderate levels of IGF-I receptors are present in cortex of the parahippocampal gyrus (**pg**). **C:** In the pituitary gland high levels of IGF-I receptors are found in the anterior lobe (**al**), while lower levels are observed in the posterior lobe (**pl**). **D:** IGF-I receptors in plexus choroideus. **E:** Coronal section through the olfactory bulb, showing high levels of IGF-I receptors in the glomerular or outer layer.

In the cerebellar cortex, high labelling was observed in the molecular layer (Figure 5A), and intermediate densities were associated with the dendate nucleus. In the midbrain IGF-I receptors were mainly seen in the substantia nigra, followed by the periaquaductal gray and red nucleus (Figure 5C). In the pons moderate levels of IGF-I receptors were associated with the pontine nuclei (Figure 5B). In the remainder of the brainstem there were no nuclei that contained higher levels of IGF-I receptors than the surrounding white matter.

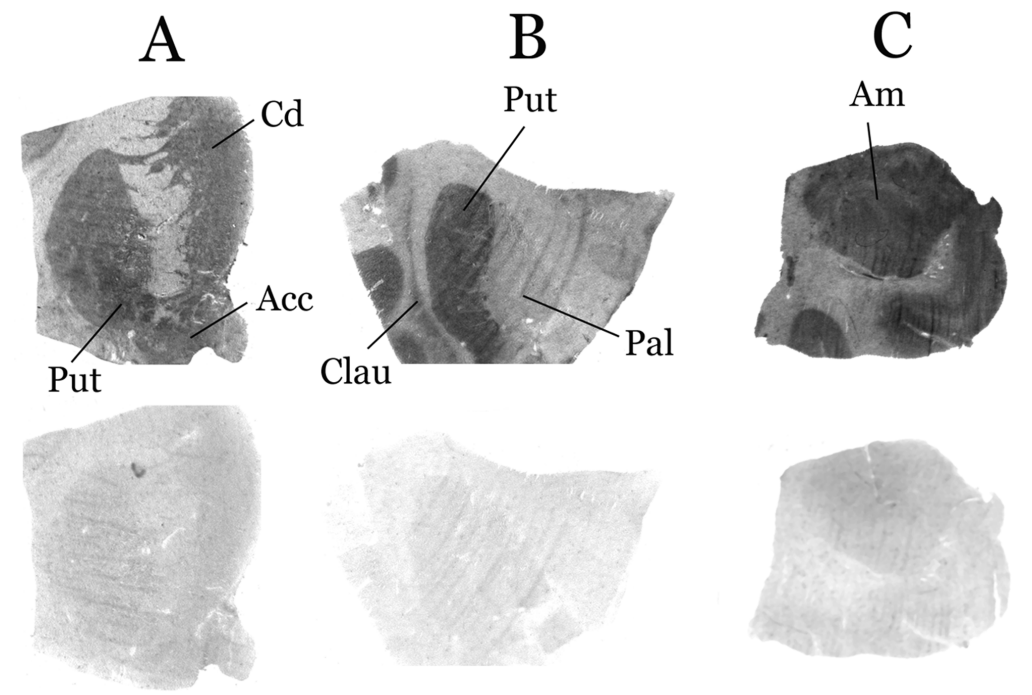
Figure 5



Autoradiographs of 0.1 nM [^{125}I]IGF-I binding in the absence = total binding (top row) and the presence of 0.5 μM unlabelled IGF-I = non-specific binding (bottom row) to microscope slide-mounted sections at the level of the cerebellum (**A**), pons (**B**), and midbrain (**C**). In cerebellar cortex the highest IGF-I receptor concentrations are seen in the molecular layer (**ml**). Intermediate concentrations are associated with the granular layer (**gl**). There is also a diffuse labelling throughout the white matter (**wm**). In the pons, moderate levels of IGF-I receptors are associated with the pontine nuclei (**pn**). In the midbrain, the highest densities are present in the substantia nigra (**sn**), and lower levels are seen in the periaquaductal gray (**pg**) and red nucleus (**nr**).

In the basal ganglia, moderate densities were found in the striatal complex including putamen, caudate nucleus and accumbens (Figure 6A, B). The thalamus and amygdala showed also moderate levels of binding, more or less homogeneously distributed over all nuclei (Figure 6C). In contrast, the globus pallidus showed low levels of binding (Figure 6B).

Figure 6



Autoradiographs of 0.1 nM [^{125}I]IGF-I binding in the absence = total binding (top row) and the presence of 0.5 μM unlabelled IGF-I = non-specific binding (bottom row) to microscope slide-mounted sections at the level of the striatum (**A** and **B**) and the amygdala (**C**). The caudate nucleus (**cd**), putamen (**put**) and accumbens (**acc**) contain comparable moderate levels of IGF-I receptors, whereas their concentration is low in the globus pallidus (**pal**). IGF-I receptors are also present in the claustrum (**clau**) and the amygdala (**am**).

Table 1 Regional distribution of the IGF-I receptors labelled by [^{125}I]IGF-I in human adult brain and pituitary gland

Region		Bound (fmol/mg protein)
Cerebral cortex	Frontal	26 ± 4
	Parietal	22 ± 3
	Temporal	23 ± 3
	Occipital	25 ± 4
	Parahippocampal	24 ± 5
	Entorhinal	21 ± 2
Caudate nucleus		28 ± 3
Putamen		26 ± 5
Accumbens		26 ± 2
Globus pallidus	Medial part	10 ± 3
	Lateral part	6 ± 1
Claustrum		21 ± 4
Thalamus	Medial nuclei	16 ± 6
	Lateral nuclei	17 ± 3
Substantia innominata		8 ± 4
Amygdala	Basal nucleus	25 ± 2
	Cortical nucleus	22 ± 3
	Lateral nucleus	17 ± 3
	Medial nucleus	18 ± 4
Hippocampus	CA1, CA2, CA3 fields	24 ± 5
	CA4	13 ± 5
	Dentate gyrus	26 ± 4
Pituitary gland	Anterior lobe	64 ± 8
	Posterior lobe	24 ± 4
Pineal gland		32 ± 6
Choroid plexus		59 ± 5
Olfactory bulb	Glomerular layer	38 ± 2
	Inner layers	8 ± 1
Cerebral white matter		9 ± 3
Corpus callosum		11 ± 2
Cerebellum	Molecular layer	36 ± 6
	Granular layer	13 ± 2
	White matter	9 ± 2
	Dendate nucleus	17 ± 3
Midbrain	Substantia nigra	26 ± 2
	Red nucleus	8 ± 1
	Periaquaductal gray	14 ± 3
Pons	Tegmentum	8 ± 1
	Pontine nuclei	22 ± 4
Medulla oblongata		8 ± 2

Slide mounted tissue sections were incubated with 0.1 nM [^{125}I]IGF-I. Autoradiograms were generated by exposing the slide-mounted tissue sections to [^{3}H]Ultrofilm for 4 days, and subsequently quantitated by computerised densitometry. Data are mean ± S.E.M. values from five individuals.

Discussion

Using ***in vitro*** receptor autoradiography, we describe the distribution of the IGF-I receptors in adult human brain and pituitary gland, as labelled by human recombinant [^{125}I]IGF-I. Under the conditions used in our study, [^{125}I]IGF-I binding occurs to IGF-I receptors and not to IGF-II receptors, (displaying lower affinity for IGF-I), or to binding proteins [4, 8, 14]. [^{125}I]IGF-I was competed for by unlabelled IGF-I more effectively than by IGF-II, which is typical for IGF-I receptors. Our study confirms that IGF-I receptors are distributed widely in the human brain [1, 25]. In contrast to these two studies it provides a more detailed mapping of the neuroanatomical structures that contain IGF-I receptors.

Overall, the distribution pattern correlated in many aspects well with the localisation of IGF-I receptors reported for adult rat brain [4, 14]. As in our study on the human brain, high binding levels were found in rat pituitary gland and plexus choroideus. In the cerebellar cortex IGF-I receptors were enriched in the molecular layer, in the hippocampus they were concentrated in the pyramidal cell layers of the CA1, CA2 and CA3 fields and dentate gyrus, and in the olfactory bulb they were mainly confined to the glomerular layer. Similarly, moderate densities of IGF-I receptors were found in the cerebral cortex, amygdala, thalamus, and the substantia nigra. However, some differences between human and rat brain were also observed. We found densities of IGF-I receptors in caudate nucleus and putamen that were similar to those in cerebral cortex, whereas in the rat these structures were reported to contain much lower densities. In rat cerebral cortex, IGF-I receptors were found to be more concentrated in layers II and VI, whereas we did not observe such a laminar distribution in human cerebral cortex.

The choroid plexus, pituitary gland and pineal gland, which are assessable to IGF-I present in serum, contained the highest concentrations of IGF-I receptors. In the pituitary gland, IGF-I receptors were predominantly found in the anterior lobe. This is not surprising, since it is well known that circulating IGF-I feeds back on the anterior lobe of the pituitary to inhibit the synthesis and release of growth hormone [28]. However, the function of IGF-I receptors in the posterior lobe is less clear. The role of the IGF-I receptors in the pineal gland and plexus choroideus remains also speculative at the present time. In the latter structure, these receptors may serve as a transport system for circulating IGFs or they may regulate the formation and composition of the cerebrospinal fluid.

Low densities of IGF-I receptors are found throughout the white matter of human and rat brain. These receptors might be located on oligodendrocytes, since IGFs are potent regulators of oligodendrocyte development [17, 18, 19]. IGF-I promotes survival of oligodendrocyte progenitors and oligodendrocytes, and stimulates the formation of myelin [2, 6, 21].

The unique distribution of IGF-I receptors in discrete regions of the adult brain suggests a functional role as trophic/survival factor or perhaps as a neuromodulator for well-defined neuronal systems, which have yet to be defined. Evidence for a specific neuronal trophic/survival function is supported by the finding that IGF-II, the predominant form of IGFs in human brain [10], which may also act through IGF-I receptors, promotes the growth and neurite formation of dopaminergic but not of serotonergic neurons in culture [15]. Evidence for a neuromodulator function is based on the reports that IGF-I increases choline acetyltransferase activity in rat neuronal cultures [11] and enhances the release of acetylcholine from rat cortical slices [23].

A better insight into the neuronal functions of IGF-I receptors may be obtained from lesion studies and perhaps from microdialysis experiments in animals. Comparative studies of IGF-I receptors in pathological conditions may also help to clarify their functional role(s) in the adult human central nervous system. For instance, because IGF-I receptors may be involved in mechanisms that promote remyelination in pathological states [12], it should be particularly interesting to examine if alterations in these receptors might play a role in human demyelinating disorders.

Acknowledgements

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